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Preliminary phytochemical and biochemical composition of different solvent extracts of red seaweed *Gracilaria corticata* from Surathkal beach, Karnataka in India

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ABSTRACT:
Seaweeds or marine algae are potential renewable resources in the marine environment. They are also one of the nature's richest sources of antioxidants such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides in large quantities. Increase in human population and illogical exploitation of resources has led to a constant search for new resources to meet the growing demand for food, medicine and energy. Seaweeds is being used as anti-oxidant, anti-coagulant anti-microbial and anti-tumor agent due to the presence of metabolites such as fatty acids, steroids, carotenoids and amino acids. Majority of their antioxidant activity is due to flavonoids, anthocyanin and coumarin. The carbohydrate content was found to be the highest in *G. corticata* followed by protein content while lipid content was found to be minimum. The main objective of the present study was to determine the biochemical composition and phytochemical composition of *G. corticata* collected from Surathkal beach of Karnataka in India.

KEYWORDS: *Gracilaria corticata*, Soxhlet apparatus, phytochemical screening, biochemical analysis.

INTRODUCTION

Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. Seaweed or marine algae are a potential renewable resource in marine environment. Nearly 6000 species of seaweed have been identified and have been grouped into green (Chlorophyceae), brown (Pheophyceae) and red (Rhodophyceae) based on their pigmentation [1].

Marine plant resources are attracting attention as a raw material for the production of phytochemicals such as alginic acid, agar-agar, carrageenan, iodine and the like, which are widely used in several industries involved in the manufacture of certain food materials, fertilizers and pharmaceuticals. Seaweeds contain various inorganic and organic substances which can benefit human health [2].

Seaweeds are the eukaryotic organisms that live in salty water and are recognized as a potential source of bioactive natural products. Algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides [3]. Many studies on *Gracilaria sp* have been reported especially on its taxonomy and habitat characteristics [4,5].

MATERIALS AND METHODS

Study area

The algal samples were collected from Surathkal beach (13 00`34.1" N lat. & 74 47`16.1" E long.)

Collection of G. corticata seaweed

Seaweeds of *G. corticata* was collected from Surathkal Beach, Karnataka, India. The collected seaweed was botanically identified by Dr. C.R.K Reddy, CSIR- Central Salt and Marine Chemicals Research Institute.

Preparation of the Extracts

The samples were brought to the laboratory in plastic bags. Few collected seaweeds were preserved for identification. Algal samples were cleaned so that epiphytes and necrotic parts were removed. Samples were rinsed with tap water to remove debris and shade dried for 5-9 days and ground thoroughly to powder in a kitchen-type blender. Fifty grams of powdered seaweeds were extracted successively using Soxhlet extractor sequentially with different solvents of increasing polarity namely: chloroform, acetone, methanol, ethanol, and water until the extract was clear. The resulting pasty extracts were stored in a refrigerator at 4°C for future use.

Qualitative phytochemical screening

The different extracts thus obtained were qualitatively tested for the presence of various phytochemical constituents [6,7].

Test for Tannins

Ferric chloride Test:

To 2 ml crude extract, a few drops of 5% ferric chloride solution was added. Formation of blue colour indicates the presence of hydrolysable tannins.

Test for Alkaloids

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Dragondroff's test:
To 2 ml of crude extract, 1% HCl was added. Boiled in water bath for 10 minutes. To this added 6 drops of Dragondroff's reagent; Reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins

Frothing test:
To 2ml of crude extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponins.

Test for glycosides
Keller-Kilani test: To 2 ml of crude extract, added 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. Poured the mixture into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interphase indicates the presence of cardiac glycosides.

Test for Flavonoids
NaOH solution test: To 2 ml of crude extract, added 2 ml of 10% NaOH solution. Yellow to orange colour indicates the presence of flavanoids.

Test for Triterpenoids
Salkowski Test:
To 2 ml of crude extract, added 1 ml of chloroform and shaken well. Then added a few drops of concentrated sulphuric acid along the sides of the test tube. A red brown colour formed at the interface indicated that the test as positive for triterpenoids.

Test for Steroids
Liebermann-Burchard reaction: To 2 ml of crude extract and to 2 ml acetic anhydride was added and a few drops of conc. H_2SO_4 was added. Blue-green ring indicated the presence of steroids.

Test for phenol
To the extract added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for resin
A few ml of the sample was mixed with water and acetone. Turbidity indicates the presence of resin.

Test for Amino acids
Ninhydrin Test:
To 1ml of the crude extract, added 5% of Ninhydrin solution. Yellow colour indicates the presence of amino acids.

Biochemical Analysis

Estimation of Carbohydrate:
The carbohydrate content was estimated by Dubois method [8]. Twenty mg of dried seaweed powder was taken and to this 1 ml of 4% phenol solution and 5 ml of concentrated sulphuric acid was added and incubated in a dark room for 30 minutes. The colour intensity developed was read in a spectrophotometer at 490 nm. Sugar content was calculated using D- Glucose as a standard and the results were expressed as mg/g sugar.

Estimation of Protein:
The protein content was estimated by Biuret method [9]. To 5 mg of dried powdered sample, added 1ml of distilled water followed by 4 ml of biuret reagent and incubated for 30 min at room temperature. Then the mixture was centrifuged for 10 min at 4000 rpm. The supernatant was collected and the optical density was measured in a Spectrophotometer at 540 nm. The protein content was calculated using BSA as standard and expressed as mg/g protein.

Estimation of Lipid:
The lipid content was estimated using chloroform-methanol mixture [10]. To 10 mg of sample, 5 ml of chloroform-methanol (2:1) mixture was added. The mixture was incubated at room temperature for 24 hours. After incubation, mixture was filtered using a filter paper. The filtrate was collected in a 10 ml pre-weighed beaker. The chloroform-methanol mixture was evaporated on a hot plate leaving a residue at the bottom of the beaker. The weight of beaker with the residue and of the empty beaker were checked to know the weight of the lipid present in the sample.

Statistical Analysis:
The samples were analyzed in triplicate. The results were expressed as mean ± standard deviation (SD) using MS Excel.

RESULTS AND DISCUSSION
Phytochemical substances such as steroids, tannins, alkaloids, saponins, glycosides, flavonoids, phenols, terpenoids, resins and amino acids were determined in various extracts (acetone, chloroform, ethanol, methanol and water) in species of algae *G. corticata* (Table 1).

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Table 1: Phytochemical analysis of the extracts of *Gracilaria corticata*

S. No.	Name of the test	G. corticata in acetone	G. corticata in chloroform	G. corticata in ethanol	G. corticata in methanol	G. corticata in water
1	**Test for steroids** LB Test	-	-	+	+	-
2	**Test for Tannins** Ferric chloride Test:	-	-	-	-	-
3	**Test for Alkaloids** Dragondroff's test:	+	+	+	+	+
4	**Test for Saponins** Frothing test:	+	-	+	+	-
5	**Test for glycosides** Keller-kilani test:	+	+	+	+	+
6	**Test for Flavonoids** NaOH solution test:	+	+	+	+	+
7	**Test for Triterpenoids** Salkowski Test:	-	-	-	-	-
8	**Test for phenol** Ferric chloride test:	+	+	+	+	+
9	**Test for resin**	+	+	+	+	+
10	**Tests for Amino acids** Ninhydrin test:	+	+	+	+	+

Table: 2 Percentage of biochemical composition in *G. corticata*

Biochemical Composition of *G.corticata*	Percentage % Mean± SD
Carbohydrate	45.46±3.24
Protein	31.73±2.63
Lipid	5.45±0.50

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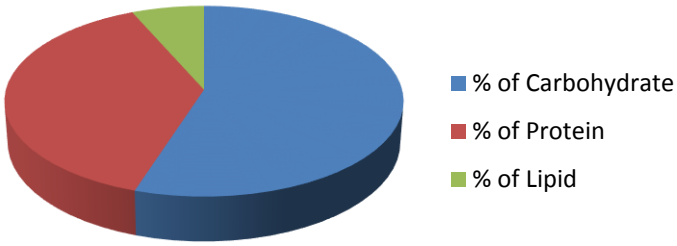

Figure:1 Graphical representation of biochemical composition in *G. corticata*

Biochemical Composition of *G. corticata*

The proximate composition of protein, carbohydrate and lipid were analyzed from seaweed *G. corticata* (Table 2)**.** The carbohydrate content was found to be the highest ranging 45.46% followed by protein content which was 31.73% while lipid content was found minimum in seaweed ranging about 5.43%(Fig.1). Shoba *et al*., had reported maximum value of carbohydrate content in Rhodophycean members than in Phaeophycean and Chlorophycean members from Kovalam coast [11]. Similarly, high protein content was reported in red alga *Hypnea valentiae* by Selvi *et al*., [12].

Fat content of seaweeds was found within the range of 1–6 g/100 g DW with high concentrations of long-chain polyunsaturated fatty acids [13,14].

CONCLUSION

The present study deals with the analysis of phytochemical and biochemical parameters. The results establish that seaweeds are rich in the carbohydrate and protein content, lipid content being far ahead. They can be a potential health food in human diets and as a source of ingredients with high nutritional value. They can provide a dietary alternative due to its nutritional value and enhance its commercial value by improving the quality of seaweed-based products. There is a need for more research seaweeds remain as an under-exploited source of health benefit molecules for food processing and nutraceutical industry.

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